IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No.:	**SJ-0014**
Inventors:	**Sorrentino et al.**
Serial No.:	**09/584,586**
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Examiner:	**Anne Marie Baker**
Group Art Unit:	**1632**
Title:	**Relationship of ABC Transport Proteins with Hematopoetic Stem Cells and Methods of Use Thereof**

DECLARATION

1. I, Brian Sorrentino am a co-inventor of the above referenced U.S. Patent Application.

2. My application teaches a method of performing *ex vivo* expansion of a gene modified hematopoietic stem cell comprising transducing a hematopoietic stem cell with a nucleic acid encoding an ABC transporter; and then culturing the gene-modified hematopoietic stem cell *ex vivo*.

3. In accordance with my invention, using an ABC transporter, stem cells can repopulate and expand in animals and humans.

4. The amplification and expansion of stem cells in our experiments was achieved for periods of time exceeding three days. Figure 2 of the application demonstrates expansion of stem cells using the present invention. The cells in this experiment were transduced and then expanded for about 12 days in order to distinguish donor cells from recipient cells. The expanding cells with the control vector shown in 2A and 2B demonstrate that by day 50, the transplanted cells are absent. In contrast 2C and 2D show an increased or expanded population of cells using the MDR vector.

5. Figure 4 further demonstrates a quantitative analysis for repopulating cells. The degree of stem cell expansion is

quantified as shown by the last six lanes on the right. In this experiment, two cultured populations of cells were mixed in equal amounts and implanted into mice. The MDR expressing cells (shown in the lower band) were the only cells to expand in the recipients, verifying that stem cells had been expanded during the culture period preceding transplantation.

6. The loss of unmodified stem cells in culture is clearly shared by mouse, Rhesus monkey and humans, The reason that stem cells are lost is that they do not tolerate *ex vivo* manipulation, that is, they either die off or differentiate. Stem cells are normally fragile and lost in culture ,unless they are modified to express an ABC transporter, such as the MDR1 gene product..

7. I am familiar with the teachings of US Patent No. 5,837, 536 (hereinafter referred to as McDonagh et al.). The McDonagh et al. reference deals with CD34 cells. Only a small proportion of the CD34+ cells in a bone marrow sample described by McDonagh et al. were stem cells (about 1 in 50,000). The vast majority of CD34+ cells are not stem cells.

8. McDonagh et al. do not teach *ex vivo* expansion of HSCs. The expansion of cells in McDonagh et al. (column 15, line 31) simply reflects the effects of hematopoietic cytokines on committed progenitor cells and does not require the MDR1 vector or reflect an expansion of true repopulating cells. Expansion of gene modified HSCs would not have been expected to occur in this example because the retroviral promoter used to express the MDR1 gene in the G1MD11 AA1.2 vector (the promoter derived from the Moloney Murine Leukemia Virus) is not expressed in primitive stem cells. As a result, expansion of HSC's would not be expected to occur because there would be no expression of the ABC transporter (MDR1) in the primitive stem cells.

9. One of skill in the art can routinely obtain great increases in the number of CD34+ cells in a culture, but the end result is that the stem cells suffer major losses. The loss of stem cells is in spite of major expansion of the CD34 cells. This concept is further exemplified in the attached reference, by Tisdale et al. entitled " Ex vivo expansion of Genetically Marked Rhesus Blood Progenitor Cells Results in Diminished Long-Term Repopulating Ability" (Blood (92) 4:1131-1141 (1998)).

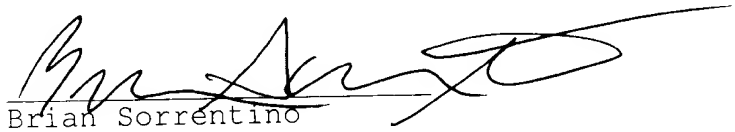
10. Hematopoietic stem cells (HSC) from mammals including primates and particularly humans, were able to be successfully transduced using methods available in the art prior to May 28, 1998. Non-human primate HSCs, a very good model of human stem cells can be transduced with retroviral vectors at frequencies up to five percent. For example, see attached, Dunbar et al. 1996 Proc. Natl. Acad. Sci.,USA, Vol 93 pp 11871-11876; and Tisdale et al. 1998 Blood 92:1131-1141 disclose successful transduction methods permitting gene transfer in HCSs with favorable transfer efficiencies.

11. Further, it has also been shown that human stem cells can be transduced with vectors at a low frequency (see, e.g. attached Dunbar et al. 1995 Blood 85: 3048-3057; Brenner, MK, 1993, Lancet,342:1134-1137). and more recent studies have shown that transduction rates of up to fifteen percent can be achieved in human patients. See attached Abonour et al. 2000 Nat.Med. 6: 652-658 and Cavazzana-Calvo et al. 2000 Science 288: 669-672.

12. The success of the claimed invention does not depend on any particular transduction method or upon a high transduction frequency. Even if a low but detectable proportion of stem cells are transduced, it is the ABC transporter mediated expansion of these cells which allows for ex vivo amplification and expansion, thereby providing a powerful tool for overcoming low gene transfer rates into stem cells. Although the transduction efficiency in mice (about 70%)is higher than the transduction efficiency in Rhesus monkeys and humans, the end result is the same. In fact, this method gives a very useful approach for overcoming the low transduction efficiency in human stem cells by allowing for this minor population to be amplified. Once transduction of stem cells occurs, the stem cells repopulate and expand, thereby increasing in number. Therefore, even a low transduction frequency will successfully increase the population of stem cells through preferential expansion and repopulation.

13. Similarities and predictability of the culturing step in light of transduction efficiencies can be obtained using a variety of different vectors. Any vector which can transduce and express an ABC transporter (such as MDR1 or BCRP or an alternative heterologous gene) will enable successful transduction and ex vivo expansion of stem cells.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.



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